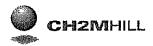
SAMOA JOINT CANNERY OUTFALL 2004 Tradewind Season EFFLUENT BIOASSAY TEST RESULTS September 2004 Sampling



16 November 2004





TECHNICAL MEMORANDUM

BIOASSAY TESTING – JOINT CANNERY OUTFALL EFFLUENT SEPTEMBER 2004 SAMPLING

Prepared For: StarKist Samoa (NPDES Permit AS0000019)

COS Samoa Packing (NPDES Permit AS0000027)

Prepared By: Steve Costa

Karen Glatzel

Date: 16 November 2004

Distribution: Carl Goldstein

United States Environmental Protection Agency, Region 9

Peter Peshut

American Samoa Environmental Protection Agency

Purpose

This memorandum presents the results of the bioassay testing of the Joint Cannery Outfall effluent sample that was collected in September 2004. The testing is required by the NPDES Permits that became effective in January 2001. This is the eighth required semi-annual test required by the current permits and the twenty-fourth test, over twenty-two semi-annual periods, conducted since testing of the Joint Cannery Outfall effluent began in 1993¹.

Study Objectives

Section D.1 of the StarKist Samoa and COS Samoa Packing NPDES Permits requires that semiannual definitive acute bioassays (96-hour static bioassays) be conducted on the cannery effluent. The purpose of these tests is to determine whether, and at what effluent concentration, acute toxicity may be detected for the combined joint cannery effluent discharge into Pago Pago Harbor.

¹ Testing was not conducted during 1999. Extra tests using two organisms were conducted in March 1995 and February 1996.

Study Approach

U.S. EPA has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests conducted in the past. All comments from U.S. EPA have been incorporated into the sampling and sample handling standard operating procedures (SOP) or have been incorporated into the procedures used by the laboratory doing the test. The comments, responses, and SOP have been documented in previous reports.

The permit conditions require that the bioassay tests be conducted with the white shrimp, *Penaeus vannami* (postlarvae). In the event *Penaeus vannami* is not available at the time of the tests, the permit specifies the substitute species, *Mysidopsis bahia*, which now has been renamed *Americamysis bahia*. For the September 2004 sampling, *Penaeus vannami* was not available and *Americamysis bahia* was used.

Effluent samples were collected from the StarKist Samoa and COS Samoa Packing facilities as 24-hour composite samples. The acute effluent bioassay test was conducted using a combined, flow-weighted, composite effluent sample made up from the effluent samples from both canneries, as allowed by the permit condition. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall to Pago Pago Harbor.

Effluent Sampling Methods

Between 1000 on 23 September 2004 and 0700 on 24 September 2004, 24-hour flow-weighted composite samples of final effluent were collected from both the StarKist Samoa and COS Samoa Packing effluent discharges. Samples were collected from the established effluent sampling sites. Detailed sampling procedures are described in the established SOP for cannery effluent sampling.

A total of eight grab samples were collected into 1-gallon plastic cubitainers at each plant. Samples were collected at approximately three-hour intervals over a 24-hour period. The samples were stored on ice or in a refrigerator until the completion of the 24-hour sampling period. After all samples were collected a flow-proportioned composite sample was prepared. The grab sample collection times, effluent flow rates, and the relative effluent flow volumes calculated from plant flow records are summarized in Table 1. The relative effluent flow volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. A chain-of-custody form for the sample was completed and sealed into a zip-lock bag and taped inside the lid of the ice chest. The sample was shipped via DHL to the testing laboratory. The chain-of-custody form and the DHL waybill are provided in Attachment I.

Table 1 StarKist Samoa and COS Samoa Packing 24-hour Composite Effluent Sample for Bioassay Testing September 2004								
Grab Sample	COS Samoa	ı Packing	StarKist	Samoa	COS Samoa Packing Percent	StarKist Samoa Percent		
Number	Sampling Date and	Effluent Flow Rate	Sampling Date and	Effluent Flow Rate	of Total Flow	Of Total Flow		
	Time	(mgd) Time (mgd)						
1	23 Sept 2004 1000	0.60	23 Sept 2004 1000	1.44	0.0254	0.0610		
2	1300	0.38	1300	2.13	0.0161	0.0901		
3	1600	0.68	1600	2.92	0.0288	0.1235		
4	1900	0.68	1900	2.50	0.0288	0.1055		
5	2200	0.56	2200	2.73	0.0237	0.1154		
6	24 Sept 2004 0100	0.56	24 Sept 2004 0100	2.53	0.0237	0.1070		
7	0400	0.56	0400	2.76	0.0237	0.1166		
8	0700	0.66	0700	1.96	0.0279	0.0829		
Total		4.68		18.97	0.1979	0.8021		
Mean		0.59		2.37	Total :	= 1.00		

Bioassay Testing Procedures

EnviroSystems, Inc. located in Hampton, New Hampshire conducted the bioassay tests. The testing procedures and results of the bioassay tests are provided in the Laboratory report included as Attachment II. This report summarizes the 96-hour acute bioassay test conducted with reference to the U.S. EPA document Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (EPA-821-R-02-012), 2002 as the sources of methods for conducting the test. The bioassay test was conducted considering and including U.S. EPA's comments on previous bioassay tests, as documented in previous reports.

The test organisms were ≤ 5 days old and the test temperature was to be held at 20 ± 1 °C, with actual temperatures ranging between 20°C and 22°C. Salinity was adjusted to 25 ppt at the start of the test and ranged during the test between 24 and

28 ppt. Demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD) had been discussed and documented in previous bioassay technical memoranda, which describe the first two tests conducted in 1993. Therefore, all of the bioassay test chambers were continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO)². The test was renewed with oxygenated sample at 48 hours.

Bioassay tests were carried out for effluent concentrations of 50, 25, 12.5, 6.25, and 3.1 percent as vol:vol dilutions in seawater. Water quality was monitored daily and parameters measured included DO, pH, salinity, and temperature. Total residual chlorine and ammonia were measured. Water quality data are provided in the Laboratory Report (Attachment II). Reference toxicant tests using sodium dodecyl sulfonate (SDS) are conducted regularly by ESI with the last one completed on 29 September 2004 and results were within one standard deviation of the historic laboratory mean.

Summary Results: Americamysis bahia Effluent Bioassay

All results from the bioassay tests are included in Attachment II. The results of the mysid bioassay tests indicate the 96-hour LC₅₀ for the effluent tested was >50 percent. The no observable effects concentration (NOEC) for the 96-hour bioassay was 50 percent and the least observable effects concentration (LOEC) was >50 percent. Results on a daily basis are summarized in Table 2.

Table 2 StarKist Samoa and COS Samoa Packing Combined Effluent Bioassay Results September 2004 Sampling						
Exposure Time Parameter						
LC 50	NOEC1	LOEC				
>50%	50%	>50%				
>50%	50%	>50%				
>50%	50%	>50%				
>50%	50%	>50%				
	t Samoa and Coined Effluent I September 200 LC 50 >50% >50%	t Samoa and COS Samoa Packir bined Effluent Bioassay Results September 2004 Sampling Parameter LC 50 NOEC ¹ >50% >50% >50% >50%				

² The high initial dilution of the actual effluent discharge (>100:1) into the Harbor, in a very short time, removes any concern about IDOD effects in the receiving water.

Discussion

Table 3 summarizes the results of the effluent bioassay tests for the samples collected in the September 2004 sampling compared to the previous bioassay tests. The LC₅₀, NOEC, and LOEC are within the range or higher than that obtained from previous tests where *Americamysis bahia* (*Mysidopsis bahia*) was used in place of *Penaeus vannami*. Figure 1 summarizes the LC₅₀ for the mysid and penaeid tests done since February 1993. Figure 2 presents the range of LC₅₀ results for mysids tests conducted since 1994. There is some variability observed in test results. The September 2004 test results along with the previous test results, for February 2004, were the highest LC₅₀ values recorded for this organism. Higher LC₅₀ values indicate lower whole effluent toxicity. There is a possible trend toward lower toxicity (higher LC₅₀) with time (see Figure 2). The trend is statistically significant at the <5% level. However, the actual significance and reason for the trend is unknown.

Conclusions

The bioassay tests for the Joint Cannery Outfall effluent for September 2004 do not indicate effluent toxicity levels to be of concern. As discussed in the previous bioassay test reports on the effluent, the time scale of the mixing of the effluent with the receiving water is on the order of seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example, an LC50 of 50%, which was observed in September 2004, corresponds to a dilution of 2:1, which is achieved within one second and within one meter of the discharge point. The discharge is located in about 180 feet of water and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and well within the initial dilution plume.

Table 3					
StarKist Samoa and COS Samoa Packing					
Combined Effluent Bioassay Results					

5 4		Parameters				
Date	Species	LC 50	NOEC	LOEC		
2/93	Penaeus vannami	4.8% ¹	3.1%	6.25%		
10/93	Penaeus vannami	15.67%	3.1%	6.25%		
2/94	Penaeus vannami	15.76%	<1.6%	1.6%		
10/94	Mysidopsis bahia ²	31.2%	25%	50%		
3/95	Penaeus vannami	14.8%	6.25%	12.5%		
3/95	Mysidopsis bahia ³	10.8%	6.25%	12.5%		
2/96	Penaeus vannami	>50%	>50%	>50%		
2/96	Mysidopsis bahia³	28.36%	12.5%	25%		
3/96	Penaeus vannami	44.4%	25%	50%		
11/96	Penaeus vannami	7.11%	3.1%	6.25%		
03/97	Penaeus vannami	39.36%	12.5%	25%		
09/97	Penaeus vannami⁴	12.3%	6.25%	12.5%		
06/98	Mysidopsis bahia ²	17.2%	6.25%	12.5%		
11/98	Mysidopsis bahia ²	15%	6.25%	12.5%		
02/00	Mysidopsis bahia²	20%	6.25%	12.5%		
08/00	Mysidopsis bahia ²	17.1%	3.1%	6.25%		
03/01	Americamysis bahia ^{2,5}	13.8%	12.5%	25%		
10/01	Americamysis bahia ^{2,6}	37.5%	25.0%	50.0%		
3/02	Americamysis bahia ^{2,6}	16.1%	12.5%	25%		
8/02	Americamysis bahia ^{2,6}	10.23%	6.25%	12.5%		
03/03	Americamysis bahia ^{2,6}	28.4%	25%	50%		
08/03	Americamysis bahia ^{2,6}	43.2%	25.0%	50.0%		
02/04	Americamysis bahia ^{2,6}	>50%	50%	>50%		
09/04	Americamysis bahia ^{2,6}	>50%	50%	>50%		

¹The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests.

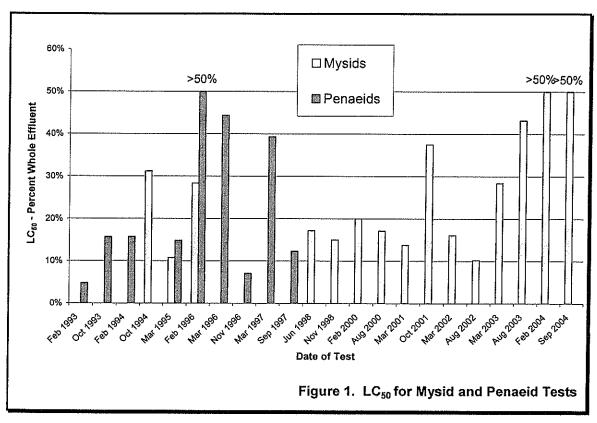
² Mysidopsis bahia used as substitutes because Penaeus vannami not available: as directed and approved

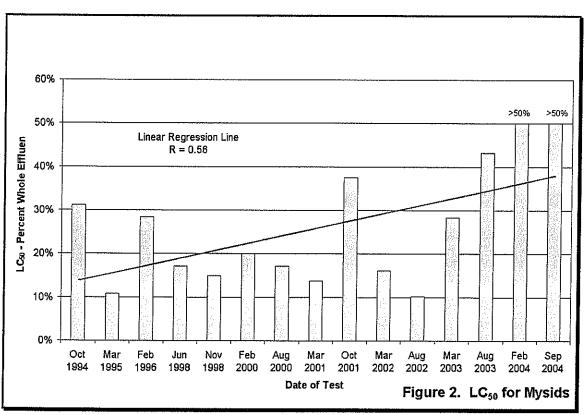
by U. S. EPA.

Mysidopsis bahia used in addition to Penaeus vannami as described in text of technical memorandums. reporting test results. Only one species is required by the permit conditions.

4Stage 1 (3 mm) *Penaeus vannami* were used for testing because older Stage 7 and 8

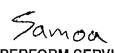
⁽⁸⁻¹⁰ mm) *Penaeus vannami* were not available. ⁵Mysidopsis bahia renamed *Americamysis bahia*. Results indicate increased toxicity because of low DO in renewal concentrations as renewal water was not aerated prior to use ⁶ Mysidopsis bahia renamed Americamysis bahia





ATTACHMENT I

Chain-of-Custody



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ATTACHMENT II

EnviroSystems, Inc. Laboratory Report

TOXICOLOGICAL EVALUATION OF A TREATED EFFLUENT: BIOMONITORING SUPPORT FOR A NPDES PERMIT OCTOBER 2004

American Samoa Joint Cannery Outfall

Prepared For

CH2M Hill, Incorporated P.O. Box 1238 Trinidad, California 95570-1238

Ву

EnviroSystems, Incorporated One Lafayette Road Hampton, New Hampshire 03842

October 2004 Reference Number CH2M-Samoa12584-04-10

STUDY NUMBER 12584

EXECUTIVE SUMMARY

The following summarizes the results of acute exposure bioassays performed from October 1-5, 2004 in support of the NPDES biomonitoring requirements of the American Samoa Joint Cannery Outfall. The 96 hour acute definitive assay was conducted using the marine species, *Americamysis bahia*.

Acute Toxicity Evaluation

Species	Exposure	LC-50	NOEC	LOEC
Americamysis bahia	24-Hours	>50%	50%	>50%
	48-Hours	>50%	50%	>50%
	72-Hours	>50%	50%	>50%
	96-Hours	>50%	50%	>50%

TOXICOLOGICAL EVALUATION OF A TREATED EFFLUENT: BIOMONITORING SUPPORT FOR A NPDES PERMIT OCTOBER 2004

American Samoa Joint Cannery Outfall

1.0 INTRODUCTION

Acute toxicity tests involve preparing a series of concentrations by diluting effluent with control water. Groups of test organisms are exposed to each effluent concentration and a control for a specified period. In acute tests, mortality data for each concentration are used to calculate (by regression) the median lethal concentration, or LC-50, defined as the effluent concentration which kills half of the test organisms. Samples with high LC-50 values are less likely to cause significant environmental impact. The acute no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) document the highest and lowest effluent concentrations that have no impact and a significant impact on the test species, respectively.

This report presents the results of an acute toxicity test conducted on an effluent sample collected from the American Samoa Joint Cannery Outfall. Testing was based on programs and protocols developed by the US EPA (2002) and involved conducting 96 hour acute static renewal toxicity tests with the marine species, *Americamysis bahia*. Testing was performed at EnviroSystems, Incorporated (ESI), Hampton, New Hampshire in accordance with the provisions of the NELAC Standards (2000).

2.0 MATERIALS AND METHODS

2.1 General Methods

Toxicological and analytical protocols used in this program follow procedures primarily designed by the EPA to provide standard approaches for the evaluation of toxicological effects of discharges on aquatic organisms, and for the analysis of water samples. See Section 4.0 for a list of references.

2.2 Test Species

Every attempt was made to acquire the species, *Penaeus vannami*, as this is the preferred organism under the Cannery's permit. ESI was unable to obtain reasonably priced *P. vannami*. Due to the exorbitant expense, the decision was made to use an alternate species, *Americamysis bahia*.

A. bahia, ≤5 days old, were from maintained at ESI. Test organisms were transferred to test chambers by large bore pipet, minimizing the amount of water added to test solutions.

2.3 Effluent and Dilution Water

The effluent sample used in the assay was identified as "JCO-04TW." Sample collection information is provided in Table 4. Upon receipt, the sample was stored at 4°C. All sample material used in the assay was warmed to 20±1°C prior to preparing test solutions. Total residual chlorine (TRC) was measured using amperometric titration (MDL 0.05 mg/L). As the effluent sample contained <0.05 mg/L, TRC dechlorination with sodium thiosulfate was not required (EPA 2002). Aliquots of the undiluted effluent sample were collected for ammonia analysis when the sample arrived and again prior to renewal. Upon arrival, the effluent sample had a salinity of 12‰. Salinity of the effluent was increased to 25‰ by the addition of artificial sea salts. Test concentrations for the assays were 50%, 25%, 12.5%, 6.25% and 3.1% effluent with a laboratory water diluent control.

The dilution water used in this assay was collected from the sea water system at ESI. The water is pumped in daily from the Hampton Estuary on the flood tide, filtered through a high volume sand filter, and stored in 3000 gallon polyethylene tanks. The water is classified as Class SA-1 by the State of New Hampshire, and has been used to culture test organisms for over 20 years. Sea water used in the assay had a salinity of 25±2‰ and a TRC of <0.05 mg/L.

2.4 Acute Toxicity Tests

The 96 hour acute static renewal toxicity test was conducted at 20±2°C with a photoperiod of 16:8 hours light:dark. Test chambers for the acute assays were 250 mL glass beakers containing 200 mL test solution in each of 5 replicates, with 10 organisms/replicate. Survival, dissolved oxygen, pH, salinity and temperature were measured daily in all-replicates. Test solutions were renewed after 48 hours using effluent from the start sample. Mysid shrimp were fed daily with <24 hour old brine shrimp.

2.5 Data Analysis

At 24 hour intervals, survival data was analyzed to assess toxicity using a program developed by Stephan (1982). LC-50 values were computed using the Spearman-Karber, Binomial, and Probit methods. If survival in the highest test concentration was >50%, LC-50 values were obtained by direct observation of the raw data. The NOEC was determined as the highest test concentration which caused no significant mortality while the LOEC was determined as the lowest concentration that did cause significant mortality.

2.6 Quality Control

As part of the laboratory quality control program, standard reference toxicant assays are conducted on a regular basis for each test species. These results provide relative health and response data while allowing for comparison with historic data sets. See Table 2 for details.

American Samoa Joint Cannery Effluent Biomonitoring Program, October 2004.

Study Number 12584. Page 4 of 9

TABLE 1. Summary of Acute Evaluation Results. American Samoa Joint Cannery Outfall Effluent Evaluation. October 2004.

Concentration	Exposure	Replicates					Mean	Standard	Coefficient
% Effluent	Liposure	A	В	С	D	E	modi.	Deviation	of Variation
Lab Control	Start	10	10	10	10	10	100%	0.000	0.00%
	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	9	10	10	10	98%	0.400	4.08%
	96-Hours	10	9	10	10	10	98%	0.400	4.08%
3.1%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	9	98%	0.400	4.08%
	72 Hours	10	10	10	10	9	98%	0.400	4.08%
	96-Hours	10	9	10	10	9	96%	0.490	5.10%
6.25%	24-Hours	10	10	10	10	9	98%	0.400	4.08%
	48-Hours	10	10	10	10	9	98%	0.400	4.08%
	72 Hours	9	9	10	10	7	90%	1.095	12.17%
	96-Hours	9	9	10	10	7	90%	1.095	12.17%
12.5%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	10	10	10	10	10	100%	0.000	0.00%
25%	24-Hours	10	10	10	9	10	98%	0.400	4.08%
	48-Hours	10	10	10	9	10	98%	0.400	4.08%
	72 Hours	10	9	10	9	10	96%	0.490	5.10%
	96-Hours	10	9	10	9	10	96%	0.490	5.10%
50%	24-Hours	10	9	10	10	10	98%	0.400	4.08%
	48-Hours	10	9	10	6	3	76%	2.728	35.89%
	72 Hours	9	8	10	6	3	72%	2.482	34.47%
	96-Hours	9	8	10	6	3	72%	2.482	34.47%

SUMMARY OF ENDPOINTS

Exposure Period	LC-50 (95% Limits)	METHOD	NOEC	LOEC
24 Hours	>50%	Direct	50%	>50%
48 Hours	>50%	Direct	50%	>50%
72 Hours	>50%	Direct	50%	>50%
96 Hours	>50%	Direct	50%	>50%

American Samoa Joint Cannery Effluent Biomonitoring Program, October 2004. Study Number 12584.

TABLE 2. Summary of Reference Toxicant Data. American Samoa Joint Cannery Outfall Effluent Evaluation. February 2004.

Concentrations Expressed as mg/L Sodium Dodecyl Sulfate

Species	Date	LC-50	Historic Mean	Number of Tests	±1 STD Deviation	±2 STD Deviations
A. bahia	09/29/04	19.4	21.2	20	3.59	7.19

TABLE 3. Summary of Effluent and Diluent Characteristics. American Samoa Joint Cannery Outfall Effluent Evaluation. October 2004.

PARAMETER	UNITS	100% EFFLUENT	50% EFFLUENT	DILUENT
Salinity - As Received	%	12		24
Salinity - After Salinity Adjustment	‰	25	25	-
pH - As Received	SU	6.48	-	8.05
pH - After Salinity Adjustment	SU	6.72	6.97	-
TRC - As Received	mg/L	‡	-	<0.05
Dissolved Oxygen - As Received	mg/L	0.9	-	-
Dissolved Oxygen - After Aeration	mg/L	-	4.8	7.6
Ammonia - As Received	mg/L as N	59	-	<0.1
Unionized Ammonia - As Received	mg/L as N	0.076	-	<0.005
Ammonia - Salinity Adjusted	mg/L as N	-	29	_
Unionized Ammonia - Salinity Adjusted	mg/L as N	-	0.115	-
Ammonia - at 48 Hours	mg/L as N	59	29	<0.1
Unionized Ammonia - at 48 Hours	mg/L as N	0.085	0.559	<0.005

TABLE 4. Summary of Sample Collection Information.

American Samoa Joint Cannery Outfall Effluent Evaluation. October 2004.

Sample		Collectio	n	Rece	ipt	Arrival
Description	Туре	Date	Time	Date	Time	Arrival Temp °C
EFFLUENT	Comp	09/23-24/04	ND	10/01/04	1510	21

COMMENTS:

^{‡ -} Analysis could not be performed due to interference.

ND - No data was recorded on chain of custody.

Summary of StarKist Samoa and COS Samoa Packing Combined Effluent TABLE 5. Bioassay Results. American Samoa Joint Cannery Outfall Effluent Evaluation. October 2004.

Date	Species	g	6-Hour Endpo	ints
		LC-50	NOEC	LOEC
02/93¹	Penaeus vannami	4.8%	3.1%	6.25%
10/93 ¹	Penaeus vannami	15.67%	3.1%	6.25%
02/941	Penaeus vannami	15.76%	<1.6%	1.6%
10/94 ¹	Americamysis bahia	31.2%	25.0%	50.0%
03/95 ¹	Penaeus vannami	14.8%	6.25%	12.5%
03/95 ¹	Americamysis bahia	10.8%	6.25%	12.5%
02/96 ¹	Penaeus vannami	>50.0%	>50.0%	>50.0%
03/96 ¹	Penaeus vannami	44.4%	25.0%	50.0%
11/96 ¹	Penaeus vannami	7.11%	3.1%	6.25%
03/97 ¹	Penaeus vannami	39.36%	12.5%	25.0%
09/97 ¹	Penaeus vannami	12.3%	6.25%	12.5%
06/98 ¹	Americamysis bahia	17.2%	6.25%	12.5%
11/98 ¹	Americamysis bahia	15.0%	6.25%	12.5%
02/00 ¹	Americamysis bahia	20.0%	6.25%	12.5%
08/00 ¹	Americamysis bahia	17.1%	3.1%	6.25%
03/01 ²	Americamysis bahia	13.81%	12.5%	25.0%
03/02 ²	Americamysis bahia	16.13%	12.5%	25.0%
08/02 ²	Americamysis bahia	10.23%	6.25%	12.5%
03/03 ²	Americamysis bahia	28.4%	25.0%	50.0%
08/03 ²	Americamysis bahia	43.2%	25.0%	50.0%
03/04 ²	Americamysis bahia	>50.0%	50.0%	>50.0%
10/04²	Americamysis bahia	>50.0%	50.0%	>50.0%

Notes:

Assays conducted by Advanced Biological Testing, Inc., Rohnert Park, California
 Assays conducted by EnviroSystems, Inc., Hampton, New Hampshire

APPENDIX A

DATA SHEETS

STATISTICAL SUPPORT

Contents	Number of Pages
Methods Used in NPDES Permit Biomonitoring Testing	1
A. bahia Acute Bioassay Data Summary	2 .
A. bahia Survival Statistics: LC-50, NOEC	4
A. bahia Organism Culture Sheet	1
Preparation of Dilutions	1
Record of Meters Used for Water Quality Measurements	1
Unionized Ammonia Calculation	1
Sample Receipt Record	1
Chain of Custody	1

METHODS USED IN NPDES PERMIT BIOMONITORING TESTING

Parameter	Method
Acute Exposure Bioassays	
Ceriodaphnia dubia, Daphnia pulex	EPA-821-R-02-012
Pimephales promelas	EPA-821-R-02-012
Americamysis bahia	EPA-821-R-02-012
Menidia beryllina, Cyprinodon variegatus	EPA-821-R-02-012
Chronic Exposure Bioassays	
Ceriodaphnia dubia	EPA-821-R-02-013, 1002.0
Pimephales promelas	EPA-821-R-02-013, 1000.0
Cyprinodon variegatus	EPA-821-R-02-014, 1004.0
Menidia beryllina	EPA-821-R-02-014, 1006.0
Arbacia punctulata	EPA-821-R-02-014, 1008.0
Champia parvula	EPA-821-R-02-014, 1009.0
Trace Metals:	
ICP Metals	EPA 200.7/SW 6010
Hardness	Standard Methods 20th Edition - Method 2340 B
Wet Chemistries:	
Alkalinity	EPA 310.2
Chlorine, Residual	Standard Methods 20 th Edition - Method 4500CLD
Total Organic Carbon	Standard Methods 20th Edition - Method 5310C
Specific Conductance	Standard Methods 20th Edition - Method 2510B
Nitrogen - Ammonia	Standard Methods 20th Edition - Method 4500NH3G
рН	Standard Methods 20th Edition - Method 4500H+B
Solids, Total (TS)	Standard Methods 20 th Edition - Method 2540.B
Solids, Total Suspended (TSS)	Standard Methods 20th Edition - Method 2540D
Dissolved Oxygen	Standard Methods 20th Edition - Method 4500-O G

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X - See: " EFFLUENT & DILUENT CHEMISTRY and WATER QUALITY DATA" sheet.
 → - AERATE FROM START!
 ◇ - "Old" water qualities (prior to renewal)

^{☆ - &}quot;New"water qualities (post renewal)

ACUTE BIOASSAY DATA SUMMARY

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[★] - See:" EFFLUENT & DILUENT CHEMISTRY and WATER QUALITY DATA" sheet.

[→] - AERATE FROM START!

^{♦ - &}quot;Old" water qualities (prior to renewal)

^{☆ - &}quot;New"water qualities (post renewal)

Title: 12584 American Samoa: A. bahie 48hr Survival

Transform: ARC SINE(SQUARE ROOT(Y)) 584ab48sv

Shapiro - Wilk's Test for Normality

D = 0.5969 W = 0.7483

Critical W = 0.9000 (alpha = 0.01 , N = 30) W = 0.9270 (alpha = 0.05 , N = 30)

Data FAIL normality test (alpha = 0.01). Try another transformation.

The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: 12584 American Samoa: A. bahie 48hr Survival

Transform: ARC SINE(SQUARE ROOT(Y)) 584ab48sv

Hartley's Test for Homogeneity of Variance Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption. Additional transformations are useless.

Title: 12584 American Samoa: A. bahie 48hr Survival

584ab48sv Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1 2 3 4 5	Lab 3.1 6.25 12.5 25 50	5 5 5 5 5 5	1.4120 1.2490 1.2490 1.4120 1.2490 0.5796	1.4120 1.4120 1.4120 1.4120 1.4120 1.4120	1.4120 1.3794 1.3794 1.4120 1.3794 1.1078

Title: 12584 American Samoa: A. bahie 48hr Survival

Transform: ARC SINE(SQUARE ROOT(Y)) 584ab48sv

Summary Statistics on Transformed Data TABLE 2 of 2

	58448												
GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %								
1 2 3 4 5 6	Lab 3.1 6.25 12.5 25 50	0.0000 0.0053 0.0053 0.0000 0.0053 0.1333	0.0000 0.0729 0.0729 0.0000 0.0729 0.3651	0.0000 0.0326 0.0326 0.0000 0.0326 0.1633	0.0000 5.2837 5.2837 0.0000 5.2837 32.9575								

Title: 12584 American Samoa: A. bahie 48hr Survival File: 584ab48sv Transform: ARC SINE(SQUARE ROOT(Y))

	Steel's Many-One	Rank Test	- Ho:	Control-	<treatme< th=""><th>ent</th></treatme<>	ent
GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1 2 3 4 5	Lab 3.1 6.25 12.5 25 50	1.4120 1.3794 1.3794 1.4120 1.3794 1.1078	25.00 25.00 27.50 25.00 20.00	16.00 16.00 16.00 16.00	5.00 5.00 5.00 5.00 5.00	

Critical values are 1 tailed (k = 5)

5847296

Title: 12584 American Samoa: A. bahia 72 & 96 Hr Survival File: 584ab7296ab Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.6257W = 0.9083

Critical W = 0.9000 (alpha = 0.01 , N = 30) W = 0.9270 (alpha = 0.05 , N = 30)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: 12584 American Samoa: A. bahia 72 & 96 Hr Survival File: 584ab7296ab Transform: ARC SI Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption. Additional transformations are useless.

Title: 12584 American Samoa: A. bahia 72 & 96 Hr Survival

Transform: ARC SINE(SQUARE ROOT(Y)) 584ab7296ab

Summary Statistics on Transformed Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Lab	5	1.2490	1.4120	1.3794
2	3.1	5	1.2490	1.4120	1.3468
3	6.25	5	0.9912	1.4120	1.2627
4	12.5	5	1.4120	1.4120	1.4120
5	25	5	1.2490	1.4120	1.3468
6	50	5	0.5796	1.4120	1.0468

Title: 12584 American Samoa: A. bahia 72 & 96 Hr Survival

Transform: ARC SINE(SQUARE ROOT(Y)) 584ab7296ab

Summary Statistics on Transformed Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Lab	0.0053 Pag	0.0729 e 1	0.0326	5.2837

		584	7296			
2	3.1	0.0080	0.0893	0.0399	6.6278	
3	6.25	0.0297	0.1723	0.0770	13.6431	
4	12.5	0.0000	0.0000	0.0000	0.0000	
5	25	0.0080	0.0893	0.0399	6.6278	
6	50	0.1055	0.3248	0.1453	31.0291	

Title: 12584 American Samoa: A. bahia 72 & 96 Hr Survival File: 584ab7296ab Transform: ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test - Ho: Control-Treatment

	Steel's Many-One	Rank Test	- Ho:	Control	<treatme< th=""><th>nt</th></treatme<>	nt
GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1 2 3 4 5	Lab 3.1 6.25 12.5 25 50	1.3794 1.3468 1.2627 1.4120 1.3468 1.0468	25.00 22.00 30.00 25.00 18.50	16.00 16.00 16.00 16.00	5.00 5.00 5.00 5.00 5.00	

Critical values are 1 tailed (k = 5)

EnviroSystems, Incorporated Organism Culture Record

Client and ESI Study Number Assay:	: American	Samoa	† 12584	
I. Organism History				
Species	Imericamysis	bakia		
Source Lab Reared	✓ Hat	chery Reared	Field Coll	ected
Hatch Date 9/28- /0/1	ay Red	ceipt Date	Age	1-5 days
Lot Number				
II. Culture Conditions		Andrews Control of the Control of th		
System			TALLES TO STATE OF THE STATE OF	
Diet Flake Food	Phy	toplankton	Trout Cho	w
	Zamana Rot	CAT. WILL BER ST. WHILE CLASS TRANSPORT OF PURPLE ST.	Catality Cat	
Prophylactic Treatments				
III. Water Quality			•	
Temperature <u>34.5</u>	°C Salinity	<u>30.1</u> ppt	DO ₂ 6.9	mg/L
рН <u>'</u> , ¬, ¬, <u>ь</u> Other			Alkalinity	mg/L
IV. Comments				
Biologist John		Da	ite	

ETTLUEN I & DILUENT CHEMISTRY and WATER QUALITY DATA

PARAMETER	100% Effluent	50% Effluent	Diluent - Lab Salt
TRC	Not Recorded - Interfe	CACO AND CONTRACTOR OF STREET	20.05
As Received - pH (SU) @ 20°C	6.48		8.05
As Received - Salinity (ppt)	12		24
As Received - Dissolved Oxygen (mg/L)‡	0,9		.7,6
As Received - Ammonia (pull)	-002 i		12597-001
Salinity Adjusted - pH (SU) @ 20°C	6.72 /	6.97	
Salinity Adjusted - Salinity (ppt)	25	25	
After-Acration - Dissolved Oxygen (mg/L)	1.3	.4,8	
Salinity Adjusted - Ammonia (pull)		-003	
48 hour Ammonia (pull) of f Sala).	-004	-005	12597 -603
48 hour pH (SU) @ 20°C C いりes	6.81	7.32	8.05

^{‡ -} Aerate prior to mixing concentrations.

PREPARATION OF DILUTIONS

STUDY: 2584		CLIENT: CH2M	HILL - America	n Samoa			·-	
	Day: 🗷	00	Day: 2	DA				·
Concentration	Vol. Eff.	Final Vol	Vol. Eff.	Final Vol	HRS	Date	Time	Initials
LAB	0	1000	0	750	0	10/1/01	1550	BB
3.1%	31	1	23.25		48_	10/3/04	1505	Trem
6.25%	625		46,87		Comm	ents:		
12.5%	125		93,75					
25%	250		167,5					
50%	500	1	376	V				

RECORD OF METERS USED FOR WATER QUALITY MEASUREMENTS

STUDY: 12584 CLIENT: CH2M HILL - American Samoa						
		war Wat	ER QUALITIES - A	bahia		
HOURS:	0	24	48 - old	48 - new	72	96
Water Quality Station #	2	2.	(. /	2	
Initials	BB	jr y	778	TP	γ.	TY
Date	10/1/04	10/2	1013	10/3	10/4/04	10/5/04

Water Quality Station #1		Water Quality Station #2		COMMENTS
DO meter#	18	DO meter#	3	
DO probe #	(0	DO probe #	11	
pH meter#	(०५७	pH meter#	1138	
pH probe #	40	pH probe #	39	
S/C meter#	455703	S/C meter#	45/30B	
S/C probe #		S/C probe #		
Salinity meter#		Salinity meter#		

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STUDY: 12584

CLIENT: CH2MHill - American Samoa PROJECT: Wastewater Treatment Plant TASK: Unionized Ammonia Calculations

		Sa	Unionized		
Day / Date	Treatment	Temperature	рH	NH ₃	NH3
		Deg C	SU	mg/L	mg/L
Day 0	Lab	21	8.05	0.1	0.005
10/01/04	As Received 100% Effluent	21	6.48	59.0	0.076
	50% Salinity Adjusted Effluent	21	6.97	29.0	0.115
					0.000
Day 2	Lab	22	8.05	0.10	0.005
10/03/04	50% Salinity Adjusted Effluent	22	7.32	59.00	0.559
	100% Salinity Adjusted Effluent	22	6.81	29.00	0.085

SAMPLE RECEIPT RECORD

EnviroSystems, Inc.
One Lafayette Road
P.O. Box 778
Hampton, N.H. 03843-0778
(603) 926-3345 • (603) 926-3521 Fax

ESIS	STUDY NUMBER: 12584 CLI	ENT: CH2M-	- Am. Sewo	α.
SAMI	PLE RECEIPT: / / DATE:/O/I/O ^L TIME:/	<u>510</u> BY:	BB]
DELI	VERED VIA: FEDEX CLIENT	□ ESI □ UPS	A OTHER DHL	
	GED INTO LAB: DATE: 10/1/04 TIME: /	510 BY:		
SAMI	PLE CONDITION:			
	CHAIN OF CUSTODY:	Ø YES	, □ NO	
	CHAIN OF CUSTODY SIGNED:	Ø YES	□ NO	
	CHAIN OF CUSTODY COMPLETE: ☐ Y	ES /	MO	٠
	SAMPLE DATE: SAMPLE TIME RECORDED: SAMPLE TYPE IDENTIFIED:	ØYES □YES ØYES	□ NO □ NO	
	CUSTODY SEAL IN PLACE:	DYES	□ NO	
•	SHIPPING CONTAINER INTACT:	Z YES	□ NO .	
- 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 - 194 -	SAMPLE TEMPERATURE (AT ARRIVAL): 21	<u>C</u> .	•
	DOES CLIENT NEED NOTIFICATION O	F TEMPERATURE? □ YES	NO ·	. . .
	SAMPLE ARRIVED ON ICE:	□ YES	√☑NO·	
СОММЕНТS:	1x Igal			
•			•	